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Understanding asthma phenotypes: the World Asthma Phenotypes (WASP) international collaboration

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ABSTRACT The World Asthma Phenotypes (WASP) study started in 2016 and has been conducted in five centres, in the UK, New Zealand, Brazil, Ecuador and Uganda.

The objectives of this study are to combine detailed biomarker and clinical information in order to 1) better understand and characterise asthma phenotypes in high-income countries (HICs) and low and middle-income countries (LMICs), and in high and low prevalence centres; 2) compare phenotype characteristics, including clinical severity; 3) assess the risk factors for each phenotype; and 4) assess how the distribution of phenotypes differs between high prevalence and low prevalence centres.

Here we present the rationale and protocol for the WASP study to enable other centres around the world to carry out similar analyses using a standardised protocol. Large collaborative and integrative studies like this are essential to further our understanding of asthma phenotypes. The findings of this study will help elucidate the aetiological mechanisms of asthma and might potentially identify new causes and guide the development of new treatments, thereby enabling better management and prevention of asthma in both HICs and LMICs.



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A protocol for the World Asthma Phenotypes study to better understand and characterise asthma phenotypes in five centres in the UK, New Zealand, Brazil, Ecuador and Uganda
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This article has supplementary material available from openres.ersjournals.com. Details of the questionnaires used in this study may be requested from the corresponding author.

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Introduction

It is now well established that there are multiple phenotypes and endotypes of asthma [1, 2]. 15 to 20 years ago it was widely believed that asthma was an allergic/atopic disease caused by allergen exposure. More recent work has shown that this model does not explain the global asthma patterns and time trends [3–8].

A recent Lancet Commission report on asthma called for greater recognition of the various phenotypes, with different underlying pathological mechanisms, often grouped under the non-specific label of asthma. The authors acknowledge the need for better research to identify different traits, such that targeted treatments can be tailored to individual characteristics [2]. Here we define asthma phenotypes as the combination of clinical, demographic and pathological characteristics of asthma and we include pathological and inflammatory endotypes as a subset within this definition.

Characterisation of asthma phenotypes is important in order to: 1) better understand the aetiological mechanisms of asthma; 2) identify specific causes; 3) guide the development of new therapeutic measures that will be effective for all asthmatic patients; and 4) enable better management and prevention of asthma in both high-income countries (HICs) and low and middle-income countries (LMICs). There are major research opportunities to address these issues [9] due to the recent development and establishment of techniques for sample collection from the target organ, *i.e.* the airways (sputum induction and nasal lavage), novel analytical approaches for biological samples (microbiome assessment and epigenetics) and emerging biostatistical methods for integrating data from multiple sources and levels (bioinformatics). There is therefore an unprecedented potential to go beyond the old atopic/non-atopic [10], eosinophilia/non-eosinophilia categorisation of asthma phenotypes.

For these reasons, the World Asthma Phenotypes (WASP) international collaboration has been formed to investigate and characterise asthma phenotypes in more depth. The study will initially be conducted in five countries, but we are publishing the background, justification and protocol here to enable other centres around the world to carry out similar analyses using a standardised protocol.

What is known about asthma phenotypes?

Until relatively recently, asthma was widely regarded, particularly in children, as an atopic disease involving allergen exposure, IgE-mediated sensitisation with a Type 2 T-helper cell (Th2) lymphocyte response and subsequent interleukin-5 (IL-5) mediated eosinophilic airways inflammation, resulting in reversible airflow obstruction and clinical symptoms (asthma). This assumption is increasingly being challenged and disproved [3, 11, 12], and there is growing interest in alternative inflammatory and non-inflammatory mechanisms for asthma [3, 13]. Studies using induced sputum have demonstrated that less than 50% of asthma cases are attributable to eosinophilic airway inflammation [3, 14]. Patients may have severe and persistent asthma in the absence of detectable eosinophilic inflammation and may experience exacerbations without an increase in eosinophilic inflammation [3]. Repeated assessments of airway inflammation have shown that the non-eosinophilic asthma (NEA) phenotype may be reproducible both in the short-term (4 weeks) and the long-term (1–5 years), but the evidence is equivocal [15, 16] and the underlying mechanisms are not fully understood.

The approaches to phenotyping asthma most commonly used to date include clinical, trigger-related, demographic and pathological factors. Most early attempts to define phenotypes were relatively one-dimensional [17] and based on simple classifications such as age of onset or atopic/non-atopic. More recently, a multi-dimensional approach has been increasingly used [17], involving statistical methods such as latent class analysis [18, 19]. However, while some recent studies have included many variables to identify asthma sub-types [20], most have continued to be based solely on clinical/demographic characteristics or pathological characteristics and few population-based studies have used multiple aspects to assemble comprehensive phenotypes. In the large Mechanisms of the Development of ALLergy (MeDALL) study, allergic multimorbidity phenotypes have been identified based on eczema, rhinitis and asthma; however, further characterisation of specific asthma phenotypes based on pathophysiological characteristics has not been performed [21].

Several features of the existing studies on asthma phenotypes are notable. First, and most importantly, nearly all of these studies have been conducted in HICs, with the exception of a small study in Brazil [22] which did not directly address the issue of phenotypes and a recent study from China [23]. The key strength of the study protocol presented here is that it is the first to undertake complex phenotype analyses in LMICs and to compare the results with those in HICs using the same methodology.

Secondly, there has been little progress on clinical wheezing phenotypes since the seminal paper of MARTINEZ *et al.* [24] in 1995, which identified the distinctions between transient wheeze, late-onset wheeze and persistent wheeze in the first 6 years of life. Most (but not all) of the clinical classifications of phenotypes are similar, in some instances with a few more added categories [25–30] (table S1). This is

perhaps not surprising, since most attempts to reclassify asthma phenotypes have only collected information on symptoms and atopy status and thus the resulting clinical phenotypes have been restricted to the categories that are possible using this limited information (atopic/non-atopic and early/late/persistent wheezing).

The need to characterise asthma phenotypes on a pathological rather than a clinical basis has long been recognised [31] and driven by the need to improve treatment, since some asthmatic patients respond to conventional treatment while others do not [2]. However, most studies have not made full use of techniques for sample collection from the target organ (sputum induction and nasal lavage), more novel methods of biomarker analysis (microbiome assessment, epigenetics and other “omics” technologies), or developing methods of biostatistical analysis (bioinformatics). Despite this, several studies have identified asthma phenotypes based on markers of airways inflammation (table S1). Perhaps the prototypical study attempting to characterise phenotypes based on such markers is that of SIMPSON *et al.* [32], which concludes that there are four inflammatory subtypes: eosinophilic asthma (EA), neutrophilic asthma, mixed granulocytic asthma and paucigranulocytic asthma. HALDAR *et al.* [33] have summarised and characterised these subtypes.

Other work has also characterised airways inflammation [34] but few studies have combined this pathological data with clinical information to produce a more comprehensive approach to describing asthma phenotypes. Until recently, the methods for sampling the airways have been invasive and therefore restricted to adult populations or opportunistic sampling of children. For this reason, there is little detailed population-based data regarding such phenotypes in children and adolescents, and most studies have been restricted to examining EA and NEA. It is also notable that few studies have found neutrophilic asthma to be important in children [35]; however, this issue has not been explored in LMICs, where non-atopic asthma appears to be relatively more important. The study protocol presented here is the first to address this issue in LMICs.

Neutrophilic asthma (often identified as $<2\%$ eosinophils and $\geq 61\%$ neutrophils in sputum [36]) appears to involve an IL-8 mediated neutrophil influx, with the subsequent neutrophil activation being a potent stimulus to increased airway hyper-responsiveness [6]. Although the relevant stimuli appear diverse (*e.g.* endotoxin, ozone, particulates and virus infection), the features are consistent with activation of innate immune mechanisms (involving Toll-like receptors and CD14) rather than (or in addition to) IgE-mediated activation of acquired immunity. There is also the potential for combined activation of both innate and allergen specific inflammatory mechanisms. This may be the case in mixed granulocytic asthma and may explain the ability of ozone and nitrogen dioxide (NO_2) to potentiate allergen-induced asthmatic responses, although the pathophysiological mechanisms are not clear.

Little is known about other phenotypes and, in particular, it is possible that in some cases NEA may involve neurogenic mechanisms which may interact with airways inflammation and remodelling [37]; however, these have rarely been studied, despite asthma being viewed predominantly as a neurological disorder until the second half of the 20th century [38].

These differences in phenotypes are important not only for understanding the aetiology of asthma, but also for asthma management. Although EA and NEA cases are generally prescribed the same asthma medication, there may be important differences in their responses to medications and other aspects of management [33]. In particular, subjects with NEA are reported to have a poor short-term response to treatment with inhaled corticosteroids (ICS), which are primarily intended to suppress eosinophilic inflammation. Furthermore, the two phenotypes have not been systematically compared with respect to their expressions of other potentially relevant biological markers (*e.g.* markers of neural involvement [39, 40] or the airways microbiome [41]).

A potential confounding factor when comparing asthma phenotypes between centres may be differences in patterns of ICS use between HICs and LMICs. In particular, in some LMICs it is unlikely that many participants will be using ICS, whilst a relatively high proportion of participants in HICs are expected to regularly be using ICS. This is important, as a number of studies in adults show that initiating ICS treatment or increasing ICS dose is associated with a significant reduction in both sputum eosinophil percentage and absolute number, and that a corresponding increase in eosinophils is observed when discontinuing ICS [15, 36, 42–45]. In some of these studies, a reduction in ICS dose has also been associated with an increase in the prevalence of the EA phenotype [15, 42]. However, change in inflammatory phenotype due to ICS treatment has not been a consistent finding and may be dependent on the population studied. For example, in a double-blind crossover study of asthmatic subjects with mild to moderate asthma, there was no significant difference in EA prevalence when comparing the same group after placebo or budesonide treatment [15]. There are also very limited data available in children and, while variation in inflammatory phenotypes over time was demonstrated in one study of 51 children with

severe asthma and 28 with mild-to-moderate asthma, there was no evidence that this was related to a change in either asthma control or ICS dose [16]. To our knowledge, no other studies have examined the effects of altering ICS treatment on inflammatory phenotypes in paediatric populations.

In summary, it is currently unclear whether asthma involves a collection of different conditions, or is a single condition with multiple mechanisms and phenotypes, or what the underlying phenotypes may be [2]. Much of the previous work was based in high-income/high-prevalence countries and has, in general, involved relatively limited information on biological markers beyond blood and sputum differential cell counts and serum proteins. Techniques for sample collection from the airways are increasingly available, as are alternative methods of analysis and new biostatistical methods for integrating data from multiple sources and levels. Thus, it is an ideal time to re-examine the identification of novel asthma phenotypes using detailed information from patients in a wide variety of geographical settings. In particular, a major shortcoming of work on asthma phenotypes to date is the scarcity of studies looking at markers of inflammation in sputum samples in LMICs and the lack of comparisons between LMICs and HICs.

The WASP international collaboration

The overarching objectives of the WASP study are as follows: 1) to better understand and characterise asthma phenotypes in HICs and LMICs, and in high and low prevalence centres; 2) to compare phenotype characteristics, including clinical severity; 3) to assess the risk factors for each phenotype; and 4) to assess how the distribution of phenotypes differs between high prevalence and low prevalence centres.

The key features of this study are the inclusion of: 1) both high and low prevalence centres from both HICs and LMICs; 2) more detailed and varied biomarker information than has been used for previous studies identifying asthma phenotypes; and 3) new bioinformatics methods for integrating data from multiple sources and levels.

Study protocol

We are conducting a multi-country study to examine asthma phenotypes in a variety of settings, including those with both high and low asthma prevalence, and from both HICs and LMICs. By identifying biological mechanisms that may or may not be common to asthma phenotypes in diverse contexts, the study aims to identify critical causal pathways relevant to both prevention and treatment.

Study centres

The study will be conducted in five centres, Bristol in the UK (the Avon Longitudinal Study of Parents and Children (ALSPAC)), Wellington in New Zealand, Salvador in Brazil, Quinde in Ecuador and

TABLE 1 Collaborating studies and centres

Centre	Characteristics	Study type
Avon Longitudinal Study of Parents and Children (ALSPAC), Bristol, UK	A high prevalence centre in a HIC	Birth cohort study with extensive detailed longitudinal information [#] New data collection at age 26–27 years
Centre for Public Health Research (CPHR), Wellington, New Zealand	A high prevalence centre in a HIC	NZACS birth cohort New data collection at age 16–20 years Cross-sectional study in children aged 12–16 years recruited from schools and the community
Social Change, Asthma and Allergy in Latin America (SCAALA), Salvador, Brazil	A high prevalence centre in a LMIC	SCAALA cohort New data collection at age 11–19 years Cross-sectional study in children aged 11–19 years recruited from three schools
Social Change, Asthma and Allergy in Latin America (SCAALA), Esmeraldas, Ecuador	A medium prevalence centre in a LMIC	Population-based cohort New data collection at age 8–12 years Cross-sectional study in children aged 12–16 years recruited from schools
Entebbe childhood asthma study, Entebbe, Uganda	A low prevalence centre in a LMIC	Cross-sectional study in children aged 12–16 years recruited from schools
HIC: high-income country; LMIC: low and middle-income country. [#] : the study website (www.bris.ac.uk/alspac/researchers/data-access/data-dictionary) contains details of all the data that are available through a fully searchable data dictionary.		

Entebbe in Uganda (table 1), with a range of prevalence levels and exposures, and a likely range of phenotype distributions. Comparing findings from HICs and LMICs is not only of interest in itself, but is relevant to the assessment of whether associations seen in HICs (*e.g.* atopy and asthma) are causal or merely incidental. This is important both globally and within Europe, since Eastern and Southern Europe include middle-income countries which currently have very low asthma prevalence, but where prevalence is increasingly rapidly as these countries become more “westernised” [46].

Recruitment

Children are the most appropriate group for this study, since they are less likely to have undergone changes due to long-term asthma medication or occupational exposure. In four centres most participants will be children and adolescents aged 12–16 years, with an age range from 8–20 years. The ALSPAC [47] participants will be in their mid-20s and thus will be older than those in the other centres; however, this centre will add critical additional information because biological samples and information on asthma status are also available for earlier years and can be related to the subsequent phenotype distribution. Furthermore, the Wellington centre will provide another high prevalence centre in a HIC and will be focused on both children and adolescents. The specific findings in children in Wellington can thus be compared with those from children in LMICs and with those in young adults in ALSPAC.

In each centre, we will recruit a minimum of 200 asthma cases and 50 non-asthmatic subjects. The focus will be on defining categories within the groups of asthmatic subjects; however, a comparison will be made with the non-asthmatic subjects in each centre in order to check that the derived asthma phenotypes do in fact distinguish asthmatic cases (of different types) from non-asthmatic cases.

The recruitment methods will differ by centre. For example, the UK centre will recruit asthmatic subjects already identified through the ALSPAC study [25]. In other centres/countries, asthmatic subjects will be recruited through follow-up with participants from existing birth or other population-based cohorts and from schools. The comparison group of non-asthmatic subjects will be chosen from the same sources (*i.e.* other participants in the ALSPAC study or other children without asthma from the same school). The inclusion and exclusion criteria for the study are shown in Table 2.

Identification of asthmatic and non-asthmatic subjects

Asthmatic cases will be identified as those with symptoms of asthma and/or use of asthma medication in the past 12 months using the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire or, in adults, the European Community Respiratory Health Survey (ECRHS) questionnaire (the same key questions occur in both). Non-asthmatic cases will be identified as having no previous or current history of asthma using the ISAAC and ECRHS questionnaires.

Data collection

Information will be collected using standardised methods and operational procedures. These will include risk factor questionnaires and clinical characterisation, as well as blood, induced sputum and nasal lavage samples. We will repeat the sputum and nasal lavage samples after approximately 3 months in a sub-group of asthmatic subjects.

TABLE 2 Inclusion and exclusion criteria

Criteria	Cases	Controls
Inclusion	Symptoms of asthma and/or use of asthma medication in the past 12 months	No previous or current history of asthma
Exclusion	Chronic disease apart from asthma [#]	Chronic disease [#]
	Pregnancy	Pregnancy
Postpone appointment	Acute exacerbation of asthma	Symptomatic respiratory infection in the last 4 weeks
	Symptomatic respiratory infection in the last 4 weeks	
[#] : people with the following chronic diseases should be excluded: chronic obstructive pulmonary disease, bronchiectasis, cystic fibrosis, any form of heart disease (including hypertension/high blood pressure, congenital heart disease or if taking heart medication), any other chronic lung or chest problems, tuberculosis, HIV.		

Risk factor and symptom questionnaire

The risk factor and symptom questionnaire will collect information about current living conditions and other risk/protective factors (most of this information has already been collected prospectively for ALSPAC). Questions on the frequency and severity of clinical symptoms, and on the use of asthma medications, will also be included based on the ISAAC Phase II (asthma management) and Phase III (environmental risk factors) modules. Additional questions on current asthma control will be added based on the Asthma Control Questionnaire (ACQ; UK/English version, Sept 2010, Mapi Research Institute) by JUNIPER *et al.* [48]. In addition to the core questionnaire, there are three optional questionnaire modules on stress, nutrition and exposure to farm animals which the investigators at each centre can choose to include.

Skin prick tests

Skin prick tests (SPTs) will be carried out according to a well-defined protocol [49, 50] with histamine and saline being used as positive and negative controls, respectively. Fifteen minutes after testing, a mean wheal size of 3 mm or greater (once reaction to the negative control has been subtracted) will be considered positive. SPT positivity will be defined as a positive SPT to at least one of a panel of at least eight commercially available allergens, including house dust mite (*Dermatophagoides pteronyssinus*), tree pollen mix, grass pollen mix, cat and dog dander, *Alternaria tenuis*, Penicillium mix, plus locally relevant allergens (table S2).

Lung function testing

Lung function testing will be conducted according to American Thoracic Society (ATS) criteria using portable ultrasonic spirometers (the EasyOne® device from ndd Medical Technologies is the recommended tool, although others are also acceptable). All spirometers will be tested regularly (by the supplier) and calibration checks will be conducted weekly by research staff when devices are in use. Lung function testing will be carried out with participants in a sitting position, with three reproducible forced expiratory manoeuvres being performed to determine the following parameters: forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), FEV₁/FVC, maximal mid-expiratory flow (MMEF) and peak expiratory flow (PEF).

Exhaled nitric oxide fraction

Exhaled nitric oxide fraction (FeNO) is an optional procedure for this study. If centres choose to include it, they should use the NObreath monitor (Bedfont Scientific Ltd, Maidstone, UK).

Blood samples

Blood samples will be collected using standardised operational procedures. Atopy will be defined on the basis of allergen specific IgE (sIgE) given the dissociation between sIgE and SPT positivity in LMICs. We recognise the possibility of cross-reactivity of IgE to common carbohydrate determinants rather than to truly allergenic proteins, especially in centres in LMICs [51, 52]. A 15-mL sample of peripheral blood will be collected from each participant and added to two 5-mL ethylenediamine tetraacetic acid (EDTA) tubes (one for a full/complete blood count and one for plasma/DNA testing) and one 5-mL serum tube for IgE analysis. Blood samples will be processed within 2–4 h and stored in aliquots at –80 °C.

Sputum induction

Sputum induction will be conducted using a standardised protocol involving saline inhalation that we have used previously [14] and which is adapted from the protocol developed by GIBSON *et al.* [53]. We will follow the guidelines of the European Respiratory Society (ERS) Task Force working group on sputum induction where possible [54]. Participants will be pretreated with 200 mg salbutamol and then administered 4.5% hypertonic saline by oral inhalation using an ultrasonic nebuliser for increasing intervals (from 30 s to 4 min), up to a total of 15.5 min. At the end, participants will produce a sputum sample into a sterile plastic container and the induced sputum will be processed within 2 h of collection. At least 100 µL of sputum plugs should be selected from the sample and the volume measured. Dithiothreitol should then be added to the selected pellet and the mixture dispersed before being filtered through a 60-µm nylon mesh. The resulting suspension should be centrifuged and the supernatant removed and stored at –80 °C for subsequent laboratory analysis. The pellet should be resuspended and cell viability (trypan blue exclusion) and total cell count determined using a haemocytometer. Cytospins of the suspension should be prepared and stained (May-Grünwald Giemsa) to identify leukocytes. A differential cell count of 200 nonsquamous sputum cells will be conducted using light microscopy. Sputum supernatants will be analysed for markers of allergic inflammation or innate immunity (*e.g.* neutrophil elastase, eosinophil cationic protein (ECP), histamine, prostaglandin D₂ (PGD₂), IL-1β, IL-13, interferon-γ (IFN-γ) and IL-8) and markers associated with airway remodelling (*e.g.* matrix metalloproteinase-9 (MMP-9), tissue inhibitor of metalloproteinase 1 (TIMP-1), transforming growth factor-β (TGF-β) and

angiopoietin). We will also measure markers of neural involvement (e.g. acetylcholine, neurokinin A, Substance P and neurotrophins). Some markers of airway remodelling (e.g. MMP-1 and TIMP-1) and neural involvement (e.g. brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF)) will be measured in serum.

The key outcome from sputum will be the differential cell count. Other markers will be measured for small groups of participants initially to establish the proportion with detectable levels. The final list of measured markers will be available from the authors.

Nasal lavage

The focus will be on the findings from the sputum (lower airways); however, we will also conduct nasal lavage (upper airways) and compare the findings, since there is still some uncertainty as to their comparability and whether nasal lavage results (which are easier to collect) can be used as a surrogate for sputum results. Nasal lavage will be conducted using atomisers to instil 2 mL of saline (or PBS) into each nostril followed by blowing strongly through the nostril to collect the sample in a sterile container [55]. Cytospin slides will be prepared and differential cell counts conducted.

Sub-study of repeatability

In each centre, a sub-group of 50 asthma cases will be sampled again after 3 months in order to assess the repeatability of the sputum and nasal lavage cell counts.

Airways microbiome and epigenetics

Where sufficient sputum sample is obtained, aliquots will be taken for microbiome and epigenetic analysis and stored at -80°C . The specific details of these analyses will be determined by the availability of tests at the time, as the methods are rapidly changing in these areas.

Gut microbiome

Stool sample collection and storage is optional (four of five centres are collecting or planning to collect these samples). This will enable a comparison of gut and airways microbiome in a future sub-study (subject to funding).

Other potential analyses

If there is sufficient material remaining after the core analyses described above, we will consider additional analyses of sputum and nasal samples, such as proteomics and mass spectrometry analyses (subject to funding).

Data analysis

Analyses will first be done separately for each centre. The findings will then be compared across centres and across groups of centres (e.g. high prevalence centres compared with low prevalence centres). A key concept here is “triangulation” (i.e. do we find comparable phenotype categories and does their distribution within the population of asthmatic subjects differ between centres?) [56]. Furthermore, do different statistical methods yield analogous phenotypes? If the derived phenotype categories are similar in the various centres, then a combined analysis will be conducted for the overall data set adjusting for centre.

We will first conduct descriptive analyses involving comparisons between asthmatic and non-asthmatic subjects. This will include information on demographic factors, lung function, asthma severity, age at onset, persistence, treatment, markers of airway inflammation and atopy. We will then use all of the information collected, clinical (including medication use), demographic, pathological and molecular, to define new asthma phenotypes by applying latent class analysis (LCA). This is a model-based clustering method for finding subtypes of related cases (latent classes) from multivariable categorical data. We will use Bayesian information criteria to decide on the number of phenotypes that provides the best fit and will explore the use of other state-of-the-art bioinformatics methods, particularly Bayesian machine learning and other “intelligent data” (i.e. “big data”) methods. We will also evaluate techniques used widely in other omic-related fields to reduce the dimensionality into informative variables (e.g. self-organising maps (SOMs)). A characteristic feature of SOMs is their ability to map potentially complex non-linear interrelationships in multidimensional datasets into visually more approachable, typically two-dimensional planes of nodes. Individuals can be assigned to nodes, allowing the evaluation of between-nodal differences in demographic and behavioural factors, biomarkers and microbiome using summary statistics, univariate tests and regression models. This work therefore has considerable potential to advance beyond the state-of-the-art and to develop new key biomarkers for incorporation into definitions of phenotypes. The large datasets will be analysed using statistical packages (e.g. Mstat, R and Stata), publically available

mining algorithms (e.g. software from SHEN *et al.* [57]), as well as new methods implemented in high-level programming languages (e.g. C++ and Perl).

Once the latent classes have been defined we will repeat the initial descriptive analyses, comparing the clinical and immunological characteristics of the various asthma phenotypes and of the non-asthmatic subjects. The comparisons of the various asthma phenotypes will include symptoms, clinical severity and medication use. We will also compare the relative prevalences of the different phenotypes across centres.

The current study is not designed to investigate the effects of changing ICS dose on sputum eosinophil percentages. Indeed, such an approach would be extremely difficult to manage in a community setting across five different countries with varying degrees of healthcare coverage. However, as we are collecting data on asthma treatment at the time of assessment for all centres (specifically data on medication in the last 12 months in the core questionnaire and frequency of short-acting bronchodilator in the past week in the ACQ) and conducting repeat assessments in 50 participants from each centre, there is opportunity within the WASP study to: 1) assess stability of phenotypes over time with no changes in treatment in all centres; 2) assess stability of phenotypes over time with patient-initiated changes in treatment if/when they occur; and 3) assess stability of phenotypes in some LMICs (*i.e.* Uganda) after introduction of ICS (which is likely to occur due to clinical and ethical obligations). This will provide an indication of whether ICS use may have biased our analyses and to what extent. In addition, we expect that a sizeable proportion of all participants in HICs will not frequently use ICS. For example, in one recent study of 77 adolescent asthmatic subjects in Wellington, New Zealand (one of the participating centres), 63% reported using ICS in the last year, whilst only 39% reported using ICS in the last two weeks [14]. In this study there were no significant differences in sputum eosinophil percentages between ICS-treated and ICS-naïve participants. Therefore, given the size of the study (1000 asthmatic subjects across five centres) and a sizeable proportion of non/infrequent ICS users in adolescents, we will have sufficient power to conduct analyses stratified by ICS use (frequent *versus* infrequent or no ICS use). This will provide further insight into whether ICS use may (partially) explain any differences in phenotype prevalence observed between centres. Finally, the absence of detectable eosinophilia in symptomatic, corticosteroid-naïve patients in several studies (including one large study of 995 ICS-naïve asthmatic subjects, in which 47% had no evidence of eosinophilia during repeated testing) suggests that NEA is a real phenomenon and not simply the result of ICS treatment [14, 58, 59]. Any bias due to ICS use may therefore be relatively small and, as noted above, we will assess this in stratified analyses.

The analyses of repeatability of sputum induction and nasal lavage will involve certain cell types and inflammatory markers in the sputum and nasal samples (eosinophils, ECP, neutrophils, myeloperoxidase (MPO), tumour necrosis factor- α (TNF- α), IL-8 and others). The initial analysis will involve comparing repeated measures based on these classifications. We will estimate the correlations of repeated measurements based on the continuous outcome of each individual marker of inflammation. The mean levels of cytokines and the more general inflammatory markers will be compared using standard t-test methods for comparing (geometric) means. Subsequently we will compare the sputum and nasal results by calculating correlations between outcomes using each method.

Study size and power

Based on previous research, we assume that each major phenotype will likely include an average of 50 cases in each centre. Therefore, in each centre, the study will have a greater than 80% power to detect a three-fold difference in eosinophil levels (or neutrophil levels) between the different phenotypes or between each phenotype and the non-asthmatic cases. It will also have a greater than 80% power to detect a two-fold difference in clinical characteristics between phenotypes (e.g. 50% compared with 25%). This is partly circular, since the phenotypes are based on these characteristics, but these estimates indicate that the study will have reasonable power to make comparisons between phenotypes and across centres. The study will also have a greater than 80% power to detect a relative prevalence ratio for a particular phenotype between two centres (e.g. 30% of cases *versus* 10% of cases). For an exposure with a population prevalence of 10%, the case-control analyses (adjusted for centre) will have a greater than 80% power to detect an odds ratio (OR) of 1.7; however, for an exposure with a population prevalence of 20% (e.g. pet ownership or parental smoking), they will have a greater than 80% power to detect an OR of 1.5. If a particular subtype of asthma which accounts for about 50% of cases (e.g. non-atopic asthma) is analysed separately, then, for an exposure with a population prevalence of 15%, the case-control analyses (adjusted for centre) will have a greater than 80% power to detect an OR of 2.0. However, for an exposure with a population prevalence of 20%, they will have a greater than 80% power to detect an OR of 1.8.

Ethics

Ethical approval for the study has been obtained from the London School of Hygiene and Tropical Medicine ethics committee (ref: 9776) and in all five study centres.

Discussion

Since the ISAAC surveys [60], the need for large international collaborative and integrative studies to understand the asthma epidemics in developed and developing countries has been clear. The WASP study aims to redefine asthma phenotypes in children, adolescents and young adults using a combination of clinical, demographic and pathological data. In particular, we expect that by combining data on inflammatory and other markers from sputum with detailed clinical information it will be possible to identify novel phenotypes. This study was developed and commenced prior to the recent Lancet Commissions Asthma Report [2]; however, our approach is highly consistent with the recommendations in this report and with previous recommendations [61].

The key and fundamentally unique feature of WASP is the inclusion of centres in LMICs as well as in HICs. This inclusion is likely to enable better characterisation of non-atopic phenotypes that may be more common in LMICs yet may also be present in a minority of asthma cases in HICs.

Given that the study centres are in a variety of settings across the world, it is essential to carefully standardise the study protocol so that we can be confident that the differences in phenotypes or distribution of phenotypes we observe are genuine and not influenced by protocol variations across study centres.

The study will initially be conducted in five centres, but we are publishing the protocol here to enable other centres around the world to carry out similar analyses using a compatible and standardised protocol. The new methods of disaggregating asthma phenotypes require large numbers of participants because the proportions of some phenotypes may be very low. As such, it will be important to collaborate to assimilate datasets for analysis by these new statistical methods [62].

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